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ABSTRACT

Introduction: Interaction of several genes is responsible for psychiatric diseases such as depression. Despite the numerous microarray studies in this field, findings are controversial and hard to conclude. Methods: Male Wistar rats were randomly selected to receive Chronic Mild Stress model for 4 weeks. Different aspects of depression were measured by forced swimming test, open field trial and sucrose preference tests in the experience group and controls. Results: Sucrose was preferred by 40% of CMS group and 80% of controls (p=0.025). Twenty percent of CMS group and 80% of controls were “active” (p=0.001). Last escape was at minute 238 for CMS group and minute 245 for controls and controls had more escape efforts. Conclusion: This paper is a preliminary report of a genomic study on animal model of depression which tries to achieve reliable results by a joint of clinical view with recent techniques. Predicted challenges in this procedure and the solutions as well as the limitations may be helpful for future researches.

Introduction

Major depressive disorder (MDD) which is characterized by a change in mood including dysphoria and anhedonia represents a spectrum of a mild hopelessness to a deep anhedonia. One in five women and one in ten men experience depressive disorders at some time during their lives (Sadock et al. 2009). The early mean age of onset, as well as the chronic pattern of the disorder emphasizes its burden. On the other hand; heterogeneity of clinical manifestations, very wide range of response to different class of medications and resistance to treatment indicate further investigations for the etiological factors and reminds the biopsychosocial approach again.

The DNA-microarray technology has come a long way since 1995 (Schena et al. 1995). Psychiatric conditions (with their special complexity and heterogeneity) seem a perfect field to make the benefits of the new techniques. The principal role for genetic factors in psychiatric diseases is proved by several family, twin and adoption studies before. Specific genes and chromosomal locations have been introduced but it is obvious that for psychiatric diseases (like depression or schizophrenia) the result of interaction of several genes are responsible, rather than a single gene (Bunney et al. 2003). The extensive range of assessment by microarray technology brings the hope to discover responsible genes and pathways and probably bases for new treatments (El and Vaugeois 2007).

Microarray studies about depression may use human samples (post mortem brain tissue, or peripheral blood) or tissues from animal models of depression. The latter will provide the opportunity to obtain sample concordance with desired clinical phenotype (Nestler et al. 2002; Willner 1984). Precious progress is gained by these studies which are mostly evaluated different or pathways of certain neurotransmitters. Serotoninergic pathway (Harris 2001; Mallo et al. 2008) and dopaminergic system (Alttoa et al. 2010; Mallo et al. 2007; Mallo et al. 2008) are among the most studied. However most of them focus on certain anatomic parts of brain according to previous evidences or theories. Prefrontal cortex, hippocampus and raphe nuclei have been the center of attention (Alttoa et al. 2010). Yet there is no constant answer and some results are controversial. Moreover, conclusion gets more compounds when studies use different models for...
depression (Uriguen et al. 2008). Despite the numerous studies in this field, different designs and alternative methods are still required.

This manuscript is a preliminary report of a genomic study on animal model of depression which tries to achieve reliable results by a joint of clinical view and recent techniques.

Methods and preliminary results

Animals

Male Wistar rats were brought into the laboratory 1 week before the start of the experiment. The animals were singly housed and were maintained on a 12 h light/dark cycle with food and water freely available. Fifteen rats were chosen randomly to enter the Chronic Mild Stress model (CMS) and 10 rats were chosen as controls. Mean age and initial weights were matched. Control animals were housed in separate cages and had no contact with the stressed animals.

The study was conducted in compliance with the Animal Protection Bill of 21 August 1997, and has been approved by the ethical Committee of Tabriz University of Medical Sciences.

Procedure

Chronic Mild Stress: CMS was used to achieve depressive-like symptoms in Wistar rats (Moreau et al. 1994; Moreau et al. 1995; Willner 1984). The protocol was carried out for four weeks as described in Table 1.

Table 1. Protocol for Chronic Mild Stress administered to male Wistar rats

<table>
<thead>
<tr>
<th>Timing</th>
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<tr>
<td>09:00</td>
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<td>R</td>
<td>Fr 2 h</td>
<td>Eb 1 h</td>
<td>R</td>
<td>rLDC</td>
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<td>20:00</td>
<td>Oi</td>
<td>F/Wd</td>
<td>Wd</td>
<td>GsC</td>
<td>rLDC</td>
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R, restraint; Oi, overnight illumination; F/Wd, food and water deprivation; Fr, food restriction; Wd, water deprivation; Eb, exposure to empty bottle; GsC, group housing in soiled cage; rLDC, reversed light dark cycle.

After the CMS which was carried out to achieve depressive-like symptoms, three tests were administered for detecting and measuring the symptoms in rats. Results of these standard tests reflect some aspects of depression. The tests are as following:

Sucrose preference: Animals were trained to consume a 1% sucrose solution following 18 h of food and water deprivation at home cage in week three. Sucrose intake was measured by change in the weight of bottles at the end of the test. Subsequently, sucrose consumption was monitored, under similar conditions, at the end of experiment. This procedure reflects the change in desire for a preferred test (sucrose) and is widely used to measure effect of treatments (Gronli et al. 2005). Sucrose was preferred by 40% of CMS group and 80% of controls (p=0.025).

Open field test: This test reflects behavioral despair as well as physical activity (Walsh and Cummins 1976). OFT was carried out at the end of experiment (90 cm square chamber, 10-cm-high). Duration of activity, spontaneity and staying at the edges were recorded. Twenty percent of CMS group and 80% of controls were “active” (p=0.001).

Forced swimming test: The Forced Swimming Test (FST) is a common behavioral test for assessing depression and efficiency of antidepressant drugs in rodents (Bielajew et al. 2003; Mallo et al. 2007). Animals are placed in acrylic glass cylinders, filled with water (23–25 °C) to a depth of 15 cm to prevent the animal from touching the bottom of the cylinder with his paws or tail, and low enough to avoid an escape through the top opening of the cylinder. The animals are thus forced to swim. Duration of activity, efforts for escape and time before immobility and passive floating are the main measures. FST was done 1 week prior and at the end of experiment. Last escape was at minute 238 for CMS group and minute 245 for controls and controls had more escape efforts.

This study is planning to select animals according to special symptoms with clinical relevance, and will probe pattern of gene expression in special anatomic parts of brain of rats, which will be described in further reports.

Discussion

The procedure will face some important steps: first of all, obtaining the tissue sample will be critical in studies like the present one. The best result will be achieved by the least lag between anesthesia and placing the brain tissue into liquid nitrogen and, of course, by expert hands. The acute stress may not influence the end results of this study as the responsible system for depressive symptoms is chronic stress and the effect is different from that of acute stress (Sadock et al. 2009).

RNA extraction from the brain (which contains considerable fat) is predicted to be the next challenge. This will need an additional step provided in the trouble shooting section of the procedure provided by manufacturer of materials for RNA extraction phase.
The study may be limited by a clinical concern, i.e. temperament. Temperament is best described as “the body’s biases in the modulation of conditioned behavioral responses to prescriptive physical stimuli” (Sadock et al. 2009). Such differences are not limited to human being and are obvious in animals as well. This may influence the results of studies, as different temperaments are very likely to be the result of difference in genome, but there is not enough evidence yet. This has not been included in previous studies as well, again because there is not adequate information to limit its effect. This problem might be solved by a very large sample size but it seems that this is not the matter of interest at present.

Conflict of interests
Authors declare that they have no conflict of interests.

Ethical issues
The protocol is approved by the regional ethical committee, Tabriz University of Medical Sciences. The method is compatible with standards as defined by the European Communities Council Directive of 24 November 1986 for animal studies.

References


